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(54) NOVEL USE AS ANTI-INFLAMMATORY AGENTS FOR BIS-HETEROCYCLIC COMPOUNDS AND PHARMACEUTICAL COMPOSITIONS THEREOF

NEUE VERWENDUNG BIS-HETEROCYCLISCHE VERBINDUNGEN UND DESSEN ZUSAMMENSETZUNGEN

NOUVELLE APPLICATION DES COMPOSES BIS-HETEROCYCLIQUES ET LES COMPOSITIONS PHARMACEUTIQUES EN TANT QU'AGENTS ANTI-INFLAMMATOIRES

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US-A- 4 970 226

- **DRUGS. EXPTL. CLIN. RESEARCH vol. 17, no. 8 , 1991 pages 389 - 393 NIXON ET AL 'novel, potent and selective inhibitors of PKC show oral anti-inflammatory activity'**
- **J.MED.CHEM. vol. 36, no. 1 , 1993 pages 21 - 29 BIT ET AL 'inhibitors of PKC'**
- **FEBS LETTERS vol. 1-2 , 1991 page 169-172 MUID ET AL 'A novel conformationally restricted PKC inhibitor, Ro 31-8425'**

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Description

Field of the Invention

This application relates to compounds which are used as anti-inflammatory agents and compositions containing such compounds as active ingredients. More particularly, the invention concerns a novel use of biologically active bis-heterocyclic compounds, e.g., bis-indoles, pharmaceutical compositions containing these compounds, and methods of producing the compounds. The novel use of the compounds relates to the anti-inflammatory properties of the disclosed bis-heterocyclic compounds, which include the bis-indoles known as topsentins, nortopsentins, dragmacidins, and their analogs and derivatives.

Background of the Invention

The prevention and control of inflammation is of prime importance to man, and much research has been devoted to development of compounds having anti-inflammatory properties. Certain methods and chemical compositions have been developed which aid in inhibiting or controlling inflammation, but additional methods and anti-inflammatory compositions are needed.

It has been found that some natural products and organisms are potential sources for chemical molecules having useful biological activity of great diversity. Marine sponges have proved to be such a source, and a number of publications have issued disclosing organic compounds derived from marine sponges. Such publications include Scheuer, P.J., Ed. (1978-1983) *Marine Natural Products, Chemical and Biological Perspectives,* Academic Press, New York; Faulkner, DJ. (1984) *Natural Products Reports* 1:551-598; Faulkner, DJ. (1986) *Natural Products Reports* 3:1-33; Faulkner, DJ. (1987) *Natural Products Reports* 4:539-576; Uemura, D., K Takahashi, T. Yamamoto, C. Katayama, J. Tanaka, Y. Okumura, Y. Hirata (1985) *J. Am. Chem Soc.* 107:4796-4798.

Indole compounds of marine origin have also been described in Moquin, C., M. Guyot (1984) *Tetrahedron Letters* 25(44):5047-5048 and Norton, R.S., R.J. Wells (1982) J. Am. Chem. Soc. 104(13):3628-3635.

Utilizing sponges as a source material and supplemented by novel synthetic production methods, new classes of biologically active compounds and new pharmaceutical compositions useful as antitumour and antiviral agents have been provided. See US-A-4866084, US-A-4895844 and US-A-4970266.

Drugs Exptl. Clin. Res. (1991) 389-393, J. Med. Chem. (1993) 21-29, and Febs. Letters (1991) 169-172, disclose bis-heterocycles linked by a pyrrolidinone ring, having anti-inflammatory properties.

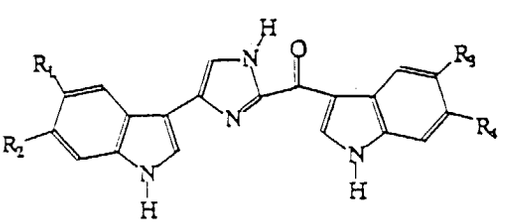
Summary of the Invention

According to the invention, anti-inflammatory activity has been found for the bis-heterocyclic compounds defined in claim 1.

An anti-inflammatory composition may be formulated, containing as active ingredient an effective amount, preferably 0.1 to 45%, especially 1 to 25%, by weight, based on the total weight of the composition, of one or more of the bis-heterocyclic compounds and a non-toxic, pharmaceutically acceptable carrier or diluent. In addition, a pharmaceutical composition can comprise at least one other anti-inflammatory compound. Such other anti-inflammatory compounds include, but are not limited to, steroidal compounds, e.g. hydrocortisone, and non-steroidal anti-inflammatories e.g., acetylsalicylic acid (aspirin), ibuprofen, acetaminophen or indomethacin.

Description of the Invention

Preferred bis-heterocyclic compounds to which this invention relates are defined in claim 2. Particularly preferred compounds of the invention are those of the formulae:

I. 

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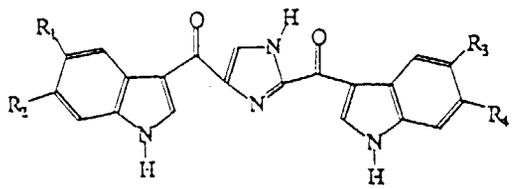

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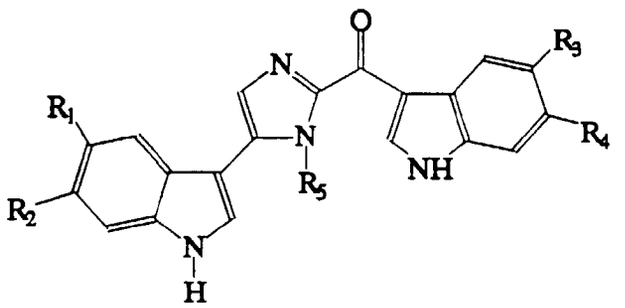

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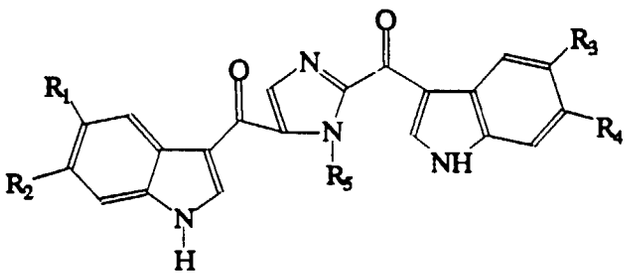

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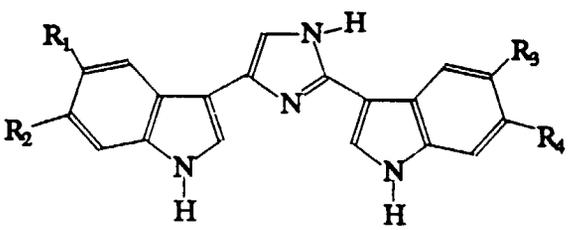

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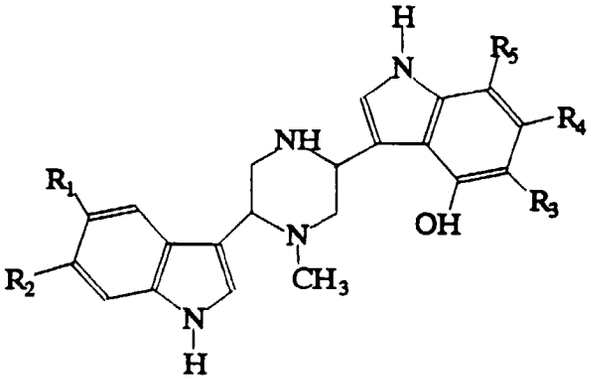
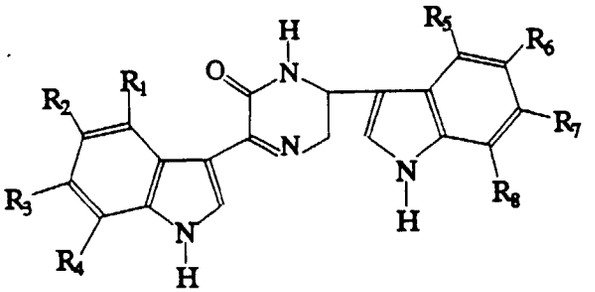
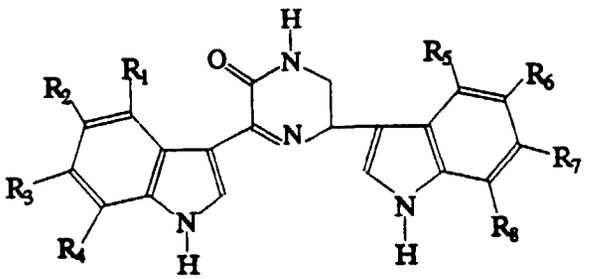


VII.



VIII.



wherein:

I(a)	R_1, R_2, R_3 = H; R_4 = OH	(Topsentin)
I(b)	R_1, R_3 = H; R_2 = Br, R_4 = OH	(Bromotopsentin)
I(c)	R_1, R_3, R_4 = H; R_2 = OH	(Isotopsentin)
I(d)	R_1, R_3 = H; R_2, R_4 = OH	(Hydroxytopsentin)
I(e)	R_1, R_2, R_3, R_4 = H	(Deoxytopsentin)
I(f)	R_1, R_2, R_4 = H; R_3 = OH	(Neotopsentin)
I(g)	R_2, R_3, R_4 = H; R_1 = OH	(Neoisotopsentin)
I(h)	R_1, R_3 = OH; R_2, R_4 = H	(Neohydroxytopsentin)
II(j)	R_1, R_2, R_3 = H; R_4 = OH	(Homocarbonyltopsentin)
III(k)	R_1, R_2, R_3, R_5 = H; R_4 = $OCOCH_3$	(Topsentin monoacetate)
III(l)	R_1, R_2, R_3=H; R_4=$OCOCH_3$; R_5=$COCH_3$	(Topsentin diacetate)
IV(m)	R_1, R_2, R_3, R_5=H; R_4=$OCOCH_3$	(Homocarbonyltopsentin mopoacetate)
V(n)	R_1, R_3 = H; R_2, R_4 = Br	(Nortopsentin A)

(continued)

V(o)	R_1, R_2, R_3 = H; R_4 = Br	(Nortopsentin B)
V(p)	R_1, R_3, R_4 = H; R_2 = Br	(Nortopsentin C)
VI(q)	R_1, R_3 = H; R_2, R_4, R_5 = Br	(Dragmacidin)
VII(r)	R_1, R_2, R_4, R_5, R_6, and R_8 = H; R_3 and R_7 = Br	(Hamacanthin A)
VIII(s)	R_1, R_2, R_4, R_5, R_6, and R_8 = H; R_3 and R_7 = Br	(Hamacanthin B)

Bis-indole compounds have been previously described as having antimicrobial, antitumor, or antiviral activity. The topsentins are disclosed in U.S. Patent No. 4,866,084. Dragmacidin and its related compounds isolated from the marine sponge of the *Dragmacidon* sp. are disclosed in U.S. Patent No. 4,895,844. Similarly, the nortopsentins have been disclosed in U.S. Patent No. 4,970,226.

The hamacanthins have not been previously disclosed. These hamacanthin compounds are exemplified herein as Formulae VII and VIII. The hamacanthins were isolated from a new species of *Hamacantha* sponge (phylum Porifera, class Demospongiae, order Poecilosclerida, family Biemnidae) collected by the Johnson-Sea-Link I manned submersible from a sand slope at a depth of 548 m off the southeast coast of Madiera (latitude 32°42.41' N, longitude 60°40.25' W). In life, the sponge was amorphous and yellow-white in color. A taxonomic voucher specimen is deposited in the Harbor Branch Oceanographic Museum (catalog no. 003:00920, DMBR no. 27-V-91-3-003). The hamacanthins have shown antifungal properties, e.g., efficacy against *Candida albicans.* Anti-inflammatory activity for the hamacanthins has now been discovered. The extraction procedure for obtaining the hamacanthins from sponge, as well as chemical characterization of the compounds hamacanthin A and hamacanthin B, are described below in Example 4.

Skilled chemists will be able to use procedures as disclosed herein and others to synthesize these compounds from available stock substances. In carrying out such operations, any suitable filtration, chromatographic, and other purification techniques include reversed phase, medium pressure, and high pressure liquid chromatography (RPLC, MPLC, and HPLC, respectively) with a suitable column as would be known to those skilled in the art, including silica gel, Sephadex LH-20, ammonia-treated silica gel, and LiChrosorb NH_2 columns. Such columns are eluted with suitable elements such as heptane, ethyl acetate, methylene chloride, methanol, isopropyl alcohol, and various combinations and ratios thereof.

One method of preparation for the compounds used for the subject invention involves extraction from marine sponges of the order Halichondrida (Phylum Porifera, Class Demospongiae) which is a problematic taxonomic group, with generic distinctions not clearly defined. Four samples used in connection with this invention have been assigned to the genus *Spongosorites,* Topsent 1896, a genus characterized by: a distinct and thick (up to 1 mm) dermal layer of smaller spicules arranged tangentially to the surface; a confused choanosomal arrangement of spicules with sporadic vague spicule tracts running parallel to the surface; bright-yellow color when alive, turning brown or black when preserved in alcohol; and two or three size categories of straight or crooked oxea. *Spongosorites* sp.1 (4-XII-84-1-22, black in alcohol) has crooked oxea and is distinguished in association with vermetids (Phylum Mollusca, Class Gastropoda); *Spongosorites* sp.3 (4-XII-84-1-23 and 23-VIII-85-1-39, tan-brown in alcohol) has fusiform straight oxea. Voucher samples are deposited in the Indian River Coastal Zone Museum of Harbor Branch Oceanographic Institution at Fort Pierce, Florida.

A current taxonomic identification of the sponge from which the compound dragmacidin was extracted is: Phylum Porifera, Class Demospongiae, Order Axinellida, Family Axinellidae, Genus *Dragmacidon,* as disclosed in U.S. Patent No. 4,895,844.

The sponge *Dragmacidon* sp. is dark brown, both alive and preserved in ethanol. The consistency is brittle and non-compressible. The ectosome is a heavy organic skin with foreign materiaL The choanosome is fibrous, with sparse dendritic-plumose spicule tracts. Spicules are trichodragmata and styles, 400-520 μm in length by 7-10 μm in width.

Identification of the sponge to the family Axinellidae and genus *Dragmacidon* is based on microscopic examination of a taxonomic voucher specimen. A similar voucher specimen is deposited at the Indian River Coastal Zone Museum (Catalog No. 003:00039), Harbor Branch Oceanographic Institution, Inc., Fort Pierce, Florida (HBOI/DBMR number 2-VI-84-3-15). The sponge in the voucher specimen is preserved in 70% ethanol with an expected shelf life of at least 30 years and is accessible to those skilled in the art for identification purposes.

The sponge *Dragmacidon* sp. was collected from southeast Grand Bahama Island (latitude 26°28.75' N, longitude 77°53.50' W) at a depth of 480 ft. on a rock and sand slope.

As disclosed herein, a novel use for the described compounds is as an agent in the control of an inflammatory response. For purposes of the subject invention, it will be understood by those of ordinary skill in the art that the terms "inflammation" and "inflammatory response" refer to any and all such inflammatory cellular and tissue reactions including, but not limited to, immune-related responses and/or allergic reactions to a physical, chemical, or biological stimulus. "Anti-inflammatory activity," as used herein, will be understood by those of ordinary skill in the art to mean biological

activity inhibiting or controlling any inflammatory response. Anti-inflammatory activity can occur by modes of action which can include, but are not limited to, lipid-mediated inflammatory responses, e.g., (i) suppression of cellular activation of phospholipase A2, either directly (as is known for the anti-inflammatory compound, manoalide) or indirectly (as is known for the anti-inflammatory compound, hydrocortisone); (ii) by inhibiting, or controlling, cyclooxygenation of arachidonic acid, similar to the action of non-steroidal anti-inflammatory drugs; or (iii) by affecting lipooxygenase products of peroxidase reactions to arachidonic acid, or by non-lipid-mediated inflammatory responses, e.g., protease-induced inflammatory responses, and the like.

Following are examples which illustrate procedures, including the best mode, for practicing the invention. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 - Preparation of Topsentin and Bromotopsentin

The frozen sample (264 g) of marine sponge, *Spongosorites ruetzleri* (Van Soest and Stentoft 1988), collected at a depth of 1149 feet at Goulding's Cay, Bahamas, was extracted twice with methanol-toluene (3:1). The combined extracts on concentration on a water bath at 30°C *in vacuo* gave as a residue (11.32 g) of crude extract which was partitioned between pentane and 10% aqueous methanol. The alcohol layer was then diluted to 30% water and extracted with CH_2Cl_2. The aqueous methanol layer was concentrated and partitioned between butanol and water. A portion (200 mg) of the *Herpes simplex* virus type 1 (HSV-1)-active, butanol-soluble fraction was dissolved in 20% aqueous methanol (1 ml) and chromatographed on a column (ID = 22 mm; height = 40 mm) packed with reversed-phase material (Amicon silica - C8, 20-45 μm). The active fraction (123 mg) was eluted with 20% aqueous methanol and purified by reversed-phase HPLC (IBM 5μ C18, 10 mm x 250 mm, 20% aqueous methanol) to yield pure topsentin I(a), 20 mg, and bromotopsentin I(b), 67 mg as yellow powder.

Topsentin, amorphous, bright-yellow solid, mp > 250°C when analyzed by conventional methods and apparatus, produced the following spectral data:

UV absorption, λ_{max} (MeOH) 208 nm (ε12,000), 246 sh (5100), 285 (4500), and 375 (4600);
IR (KBr) 3395, 3275,1635, 1590, 1530, 1455, 1270, 1165, 1115, 1095, 1005, and 876 cm^{-1};
^1H NMR (360 MHz, DMSO-d_6 + 1% TFA-H) 6.841 (1H, dd, = 8.6, 1.8 Hz), 6.997 (1H, d, J = 1.8 Hz), 7.201 (2H, m), 7.523 (1H, d, = 7.9 Hz), 7.990 (1H, d, J = 7.6 Hz), 8.041 (1H, d, J = 8.6 Hz), 8.155 (1H, d, J = 2.8 Hz), 8.159 (1H, s), 8.487 (1H, d, J = 3.2 Hz), 11.762 (1H, s), 12.355 (1H, d, J = 2.2 Hz);
^{13}C NMR (90 MHz, DMSO + 1% TFA-H) 98.11 (d), 102.72 (s), 113.12 (d), 113.95 (s), 116.00 (d), 118.67 (s), 119.46 (d), 120.50 (d), 122.02 (d), 122.44 (d), 124.27 (s), 125.74 (d), 131.11 (s), 136.53 (s), 137.78 (d), 138.33 (s), 141.23 (s), 155.25 (s), 171.5 (s);
EIMS 342 (100%, $C_{20}H_{14}N14O_2$, M^+), 209 (39, $C_{12}H_7N_2O$), 183 (28, $C_{11}H_9N_3$), 171 (17, $C_{10}H_7N_2O$), 160 (145, $C_9H_7N_2O$), 133 (65, C_8H_7NO), and 105 (15).

Bromotopsentin, yellow crystals, m.p. 296-7°C, when analyzed by conventional methods and apparatus, produced the following spectral data:

UV absorption, λ_{max}, (MeOH) 209 nm (E13,000), 236 (9700), 287 (5000), and 374 (5800);
IR (KBr) 3400-3100, 2255, 2120, 1635, 1590,1520, 1445,1265, 1230, 1165, 1028, 1005, and 875 cm^{-1};
^1H NMR (360 MHz, $CDCl_3$:CF_3COOH: 1:1) 7.098 (1H, dd, J = 8.6, 2.4 Hz), 7.193 (1H, d, J = 2.4 Hz), 7.227 (1H, dd, J = 8.6, 1.8 Hz), 7558 (1H, dd, J = 8.6 Hz), 7.668 (1H, d, J = 1.8 Hz), 7.824 (1, s), 7.927 (1H, d, J = 3 Hz), 8202 (1H, d, J = 8.6 Hz), 8.371 (1H, d, J = 3 Hz), 9.272 (1H, brs), 10.409 (1H, brs);
^{13}C NMR (90 MHz, $CDCl_3$:CF_3COOH : 1:1) 101.6 (d), 103.7 (s), 116.7 (d), 117.0 (s), l17.5 (d), 118.2 (d), 119.6 (s), 1215 (d), 122.6 (s), 125.2 (s), 125.5 (d), 127.7 (d), 128.0 (d), 135.0 (s),139.7 (s), 140.5 (s), 140.8 (d), 141.7 (s), 155.0 (s), 172.4 (s);
EIMS 422/420 (40%, $C_{20}H_{13}BrN_4O_2$, M^+), 394/392 (1.3, $C_{19}H_{11}BrN_3O_2$), 342 (13, M^+-Br), 289/287 (6%, $C_{12}H_7BrN_3O$), 263/261 (100, $C_{11}H_8BrN_3$), 223/221 (13, $C_9H_6BrN_2$), 209/207 (9.5, C_9H_6BrNO), 182 (15, 261-Br), and 133 (94, C_8H_7NO).

Example 2 - Preparation of a Deoxybromotopsentin and Other Related Analogs and Derivatives

Frozen sponge sample of *Spongosorites* sp.3 collected at Goulding's Cay, Bahamas at -229 m was homogenized and steeped repeatedly in methanol and 10% toluene followed by methanol. The alcohol layer was concentrated and re-partitioned between 1-butanol and water, and the butanol-soluble fraction was vacuum chromatographed over RP material (Amicon, silica gel C18, 20-45 μm) using 20% aqueous methanoL The yellow fraction was then subjected twice to RP-HPLC (C18, 5 μm, 20% water in MeOH) to give bromotopsentin I(b) and 4,5-dihydro-6"-deoxybromo-

topsentin. 4,5-dihydro-6"-deoxybromotopsentin is a yellow powder with the following spectral data:

$[\alpha]^{24}D$ 198° (*c* 2.0, MeOH)
UV (MeOH) λ_{max} nm 328 (ε5700), 274 (8800), 214 (34,000), 198 (29,500);
IR (KBr) 3630, 3390, 3280, 2920, 2860, 1664, 1570, 1450, 1420, 1332, 1240, 1160, 1120, 1100, 1020, 950, 805, and 750 cm^{-1};
LREIMS *m/z* (rel. intensity) 406 (95), 404 (100), 378 (41), 376 (39), 326(10), 298 (6), 297 (7), 291 (10), 289 (9), 235 (6), 233 (6), 210 (12), 208 (10), 197 (10), 195 (10), 189 (5), 156 (12), 155 (19), 144 (28), 130 (14).
^1H and ^{13}C NMR collected data also supported the structure given above.
Calcd for $C_{20}H_{13}{}^{79}BrN_4O$: 404.0272 (M - 2H). Found 404.0300 (HREIMS).

The conversion of bromotopsentin to topsentin; the preparation of 3-(hydroxyacetyl)indole, 3-chloroacetyl-6-(benzyloxy)indole, and 3-hydroxyacetyl-6-(benzyloxy)indole as synthons; the synthesis directly from (hydroxylacetyl)indoles of O-benzyltopsentin, O-benzylisotopsentin, O,O'-dibenzylhydroxytopsentin, and deoxytopsentin **I(e)**; the preparation of compounds **I(e)** and O-benzyltopsentin, O-benzylisotopsentin, and O,O'-dibenzylhydroxytopsentin from isolated glyoxal intermediates; the conversion of O-benzyltopsentin to topsentin **I(a)**; the conversion of O-benzylisotopsentin to isotopsentin **I(c)**; the synthesis of hydroxytopsentin **I(d)** from 3-hydroxy-acetyl4-(benzyloxy)-indole; preparation of 3-chloroacetyl-5-benzyl(oxy)indole; the preparation of 3-hydroxyacetyl-5-(benzyloxy)indole; synthesis of neohydroxytopsentin **I(h)**; and synthesis of neotopsentin **I(f)** and neoisotopsentin **I(g)** are described in U.S. Patent No. 4,866,084, which has been incorporated herein by reference. Topsentin monoacetate **III(k)** and topsentin diacetate **III(l)** can also be converted from topsentin or its analogs by methods well known and readily available to those skilled in the art.

Example 3 - Isolation of Nortopsentin A, B, & C

The sponge *Spongosorites* sp. (80 g), collected at the depth of 630 ft. off Chub Cay, Bahamas, on August 26, 1985, was lyophilized and extracted with methanol-toluene (3:1). The extract was evaporated to dryness and partitioned between ethyl acetate and water. The water soluble fraction was further partitioned with butanoL The combined ethyl acetate and butanol fractions were chromatographed on a Hibar Lichrosorb NH_2 column using HPLC with $CHCl_3$-MeOH (5:1) as elution solvent to yield a semi-purified compound, nortopsentin B (3 mg).

Sponge of the genus *Halichondria* (830 g) was collected at the depth of 1512 ft. off Nassau, Bahamas, on March 15, 1987. The frozen sponge was extracted with 1.5 1 of methanol four times. The extracts were combined and concentrated under reduced pressure to give a 400 ml of water suspension, which was then extracted with ethyl acetate (300 ml x 3). The resulting ethyl acetate fraction was evaporated to dryness to yield a crude fraction (12.02 g). It was found that the majority of the components in this fraction was topsentin and bromotopsentin.

A two-phase solvent system was generated by mixing heptane, ethyl acetate, methanol, and water in a ratio of 4:7:4:3. The crude fraction (12.00 g) was partitioned between 150 ml of the upper phase solvent and 300 ml of the lower phase solvent. The resulting lower layer fraction was extracted with 150 ml of the upper phase solvent three more times. The combined upper layer fractions were evaporated to dryness (5.75 g) and dissolved in 50 ml of the upper phase solvent, The solids were filtered off and the eluant was evaporated to dryness (4.46 g). The residue was dissolved again in 30 ml of the upper phase solvent. After removal of the insoluble material and evaporation of the solvent, 2.75 g of a solid was obtained.

This solid was further fractionated by using centrifugal countercurrent chromatography with two different solvent systems consisting of heptane/ethyl acetate/methanol/water in ratios of 4:7:4:3 and 5:7:4:3. A fraction containing nortopsentin A and a mixture of nortopsentin B and C along with topsentin (400 mg) and bromotopsentin (540 mg) were obtained. Nortqpsentin A (250 mg) was purified by HPLC on a Hibar NH_2 column (10 x 250 mm), using 5:1 chloroform/methanol as eluant. Preparative TLC (Kieselgel $60F_{264}$, 2 mm thickness, ethyl acetate) afforded a pure nortopsentin C (200 mg) and a fraction containing nortopsentin B. Pure nortopsentin B (250 mg) was finally recrystallized from ethyl acetate/chloroform.

Example 4 - Extraction and Isolation of Hamacanthins

A freshly thawed *Hamacantha* sp. sponge (223 g, wet weight) was extracted three times with EtOH. The concentrated extract was then partitioned between EtOAc and H_2O. The EtOAc soluble fraction (1.2 g) showed activity against *C. albicans* (MIC = 3.1 μg/ml; RPMI-1640 growth medium). This active fraction was chromatographed on Si gel (Kiesel gel 60 H) using CH_2Cl_2/MeOH step gradient. The antifungal active fraction (70 mg, *C. albicans* MIC = 1.6 μg/ml; RPMI-1640) that eluted with 2% MeOH/CH_2Cl_2 on HPLC (Si gel, 5μ, 250 x 10 mm) with 2.5% MeOH/CH_2Cl_2 gave hamacanthin A and hamacanthin B as the active components.

Hamacanthin A. Pale yellow power (22 mg, 0.011% from frozen sponge); $[\alpha]^{24}D$ 84° (c=0.1 MeOH); ir (neat) 3225

broad, 2294, 1672, 1585, 1437 cm^{-1}; uv λ max (MeOH) 219 nm (ε 76500), 280 (20600), 325 (13300); hrfabms (nitrobenzyl alcohol) *m/z* 486.9605, Δ 1.2 mmu for $C_{20}H_{15}{}^{79}Br^{81}BrN_4O$ (M+H); lrfabms (nitrobenzyl alcohol) *m/z* (rel. int.) 489 (18), 487 (36), 485 (21), 460 (11), 307 (100), 289 (55), 245 (98). NMR data is shown in Table 1.

Hamacanthin B. Pale yellow powder (27 mg, 0.014%); $[\alpha]^{24}{}_D$ 172° (c=0.1 MeOH); ir (neat) 3250 (broad), 2925, 1672, 1585, 1437 cm^{-1}; uv λ max (MeOH) 219 nm (ε 76500), 280 (20600), 325 (13300); hrfabms (nitrobenzyl alcohol) *m/z* 486.9606, Δ 1.3 mmu for $C_{20}H_{15}{}^{79}Br^{81}BrN_4O$ (M+H); lrfabms (nitrobenzyl alcohol) *m/z* (rel. int.) 489 (40%), 487 (88), 483 (43), 460 (13), 429 (17), 307 (100), 289 (60), 245 (60). NMR data is shown in Table 1.

Table 1.

^{13}C- and ^{1}H NMR data[a] for hamacanthin A and hamacanthin B in DMSO-d_6					
	Hamacanthin A				Hamacanthin B
	^{13}C[b]	^{1}H[c]	HMBC (^{1}H)	^{13}C	^{1}H
1		8.78 (d, 1.0)			8.49 (t, 1.5)
2	157.4[d]		H1	157.2 s	
3	157.6 s		H5, H2'	157.0 s	
5	53.4 t	4.05 (dd, 16.2, 5.1) 4.10 (dd, 16.2, 8.5)	H1, H6	53.6 d	5.25 (dd, 9.5, 4.8)
6	46.1 d	4.98 (ddd, 85, 5.1, 1.0)	H1, H5, H2"	43.2 t	3.47 (ddd, 12.9, 9.5, 1.0) 3.61 (ddd, 12.9, 4.8, 1.5)
1'		11.59 (d, 2.7)		11.62 s	
2'	132.6 d	8.41 (d, 2.7)		132.7 d	8.41 (s)
3'	111.0 s		H1', H2'	111.0 s	
3a'	125.0 s		H2', H5', H'	125.0 s	
4'	124.1 d	8.29 (d, 85)		124.1 d	8.30 (d, 8.7)
5'	123.2 d	7.20 (dd, 8.5, 2.0)	H7'	1233 d	7.17 (dd, 8.7, 1.6)
6'	114.7 s		H4'	114.8 s	
7'	114.2 d	7.62 (d, 2.0)	H5'	114.1 d	7.63 (d, 1.6)
7a'	137.0 s		H2', H4'	136.9 s	
1"		11.15 (d, 2.3)		11.12 s	
2"	1245 d	730 (d, 2.3)	H6	123.6 d	7.28 (s)
3"	113.1 s		H5, H6, H1"	114.8 s	
3a"	124.6 s		H2", H5", H7"	125.0 s	
4"	120.7 d	7.66 (d, 8.5)		120.8 d	7.65 (d, 8.6)
5"	1215 d	7.13 (dd, 85, 2.0)	H7"	121.4 d	7.13 (dd, 8.6, 1.6)
6"	114.7 s		H4"	113.9 s	
7"	114.2 d	756 (d, 2.0)	H5"	114.2 d	7.59 (d, 1.6)
7a"	137.2 s		H1", H2"	137.2 s	

[a]Table entries are chemical shift, ppm from solvent (multiplicity, J in Hz).

[b]Assignments based on APT, DEPT, and HMQC experiment.

[c]Assignments based on COSY and TOCSY experiments.

[d]Assignment based on a SINEPT experiment.

Example 5 - Anti-Inflammatory Properties of the Bis-Indole Compounds

The anti-inflammatory activity of a particular compound can be demonstrated by two standard assays, one *in vivo* and one *in vitro.* These assays are commonly employed by those skilled in the art and are accepted as indicative of anti-inflammatory activity in humans. These assays are described below.

A. Mouse ear anti-inflammatory assay. The test compound and a known inflammatory agent, phorbol myristate acetate (PMA), are topically applied simultaneously to the left ears of mice. Three hours and 20 minutes following application, the mice are sacrificed. Both left and right ears are removed and standard-sized bores taken. Edema (inflammation) is measured as the difference in weight between left and right ears (Van Arman, C.G. [1974] *Clin. Pharmacol. Ther.* 16:900-904).

B. *In vitro* inactivation of bee venom phospholipase A2. Phosphatidylcholine dipalmitoyl (labeled and unlabeled) is used as the substrate in monomolecular form. Test compounds are preincubated with the enzyme (25 units/0.5 ml) for

1 hour at 41°C The reaction is initiated by the addition of an aliquot of the drug-enzyme mixture to the substrate (0.68 μmoles/0.5 ml) and allowed to continue for 15 seconds. The reaction is terminated and the hydrolysis product is measured via scintillation counting. For screening, the test compounds (in methanol or DMSO) are added to the enzyme at a standard bench concentration of 5 mg/ml, for an enzyme-incubation concentration of 80 μg/ml, and a final concentration of 0.8 μg/ml. Assays are done in triplicate and results are averaged and compared to a vehicle control rate of hydrolysis.

The bis-heterocycle compounds of the subject invention, e.g., the bis-indole compounds, show significant anti-inflammatory properties. When screened for the ability to reduce edema in mouse cars caused by application of a known inflammatory agent, phorbol myristate acetate, topsentin was found to have greater potency than to the known anti-inflammatories hydrocortisone, indomethacin, and manoalide (see Table 2).

Table 2.

Relative potency of topsentin, manoalide, Indomethacin, and hydrocortisone in the topical inhibition of PMA-induced mouse ear edema	
Compound	**ED_{50} (μg/ear)**
Hydrocortisone	20
Indomethacin	250
Manoalide	100
Topsentin	15

In addition, bis-indole compounds were tested for percent inhibition of PMA-induced edema. Topsentin, bromotopsentin, dragmacidin, nortopsentin A, and nortopsentin C displayed significant potency (Table 3). Topsentin monoacetate and diacetate showed moderate activity in this assay.

Table 3.

Percent inhibition of PMA induced edema in mouse ears by topsentin and analogs			
Compound Name	**Compound Number**	**Dose**	**% Inhibition of edema**
Topsentin	HB 18	50 μg/ear	70.6
Bromotopsentin	HB 19	50 μg/ear	75.4
Topsentin monoacetate		50 μg/ear	45.8
Topsentin diacetate		50 μg/ear	42.6
Dragmacidin	HB 6	50 μg/ear	64.0
Nortopsentin A	HB 127	50 μg/ear	98.1
Nortopsentin B	HB 128	50 μg/ear	38.2
Nortopsentin C	HB 129	50 μg/ear	70.1
Hamacanthin A	HB 191	50 μM	50.0
Hamacanthin B	HB192	50 μM	34.0

Topsentin also proved to be capable of the inactivation of bee venom phospholipase A2 (Table 4). Hydrocortisone and indomethacin were inactive at concentrations up to 1 mM.

Table 4.

Relative potency of topsentin, manoalide, indomethacin, and hydrocortisone in the inactivation of bee venom phospholipase A2	
Compound	**IC_{50}**
Hydrocortisone	> 1 mM
Indomethacin	> 1 mM
Manoalide	0.05 μM
Topsentin	0.5 μM

Bis-indole compounds of the subject invention were tested at a final concentration of 1 μM for their percent inactivation of the bee venom phospholipase A2. The results of these tests are shown in Table 5.

Table 5.

Percent inactivation of bee venom phospholipase A2 by topsentin and analogs			
Compound Name	**Compound Number**	**Final Concentration**	**% Inactivation**
Topsentin	HB 18	1 μM	67
Bromotopsentin	HB 19	1 μM	33
Topsentin monoacetate		1 μM	42
Topsentin diacetate		1 μM	32
Dragmacidin	HB 6	1 μM	27
Nortopsentin A	HB 127	1 μM	30
Nortopsentin B	HB 128	1 μM	27
Nortopsentin C	HB 129	1 μM	26
Hamacanthin A	HB 191	1.6 μM	37
Hamacanthin B	HB 192	1.6 μM	32

In consideration of the data presented, the subject bis-indole compounds have been shown to have potent anti-inflammatory characteristics with unique clinical applications. Their mechanism of action appears to be the consequence of inactivation of phospholipase A2.

Dose-response curves for topsentin and bromotopsentin were generated and are shown as Figures 1, 2, and 3. Specifically, the dose-response curve for topsentin, measured as percent inactivation of bee venom phospholipase A2 (Figure 1) shows up to 80% inactivation at a final concentration of approximately 2 μM. In the mouse ear edema inhibition assay, a dose of about 12 μg/ear of topsentin achieved nearly 50% inhibition of edema, and doses of 100 μg/ear showed more than 90% inhibition of edema (Figure 2). Similarly, percent inhibition of mouse ear edema by bromotopsentin ranged from about 20% inhibition for a dose of approximately 25 μg/ear to about 75% inhibition at a dose of 50 μg/ear.

Example 6 - Formulation and Administration

The compounds of the invention are useful for various non-therapeutic and therapeutic purposes. It is apparent from the testing that the compounds of the invention are effective for immunomodulation, antiviral activity, and for controlling tumor growth. The compounds can be used to inhibit unwanted viral growth in the work areas of virology labs. Also, the compounds can be used as ultraviolet screeners in the plastics industry since they effectively absorb UV rays. As disclosed herein, they can be used therapeutically for treating tumors, or as immunomodulatory or antiviral agents in animals and humans.

Therapeutic application of the new compounds and compositions containing them can be contemplated to be accomplished by any suitable therapeutic method and technique presently or prospectively known to those skilled in the art. Further, the compounds of the invention have use as starting materials or intermediates for the preparation of other useful compounds and compositions.

The dosage administration to a host in the above indications will be dependent upon the identity of the infection, the type of host involved, its age, weight, health, kind of concurrent treatment, if any, frequency of treatment, and therapeutic ratio.

The compounds of the subject invention can be formulated according to known methods for preparing pharmaceutically useful compositions. Formulations are described in detail in a number of sources which are well known and readily available to those skilled in the art. For example, *Remington's Pharmaceutical Science* by E.W. Martin describes formulations which can be used in connection with the subject invention. In general, the compositions of the subject invention will be formulated such that an effective amount of the bioactive compound(s) is combined with a suitable carrier in order to facilitate effective administration of the composition.

In accordance with the invention, pharmaceutical compositions comprising, as active ingredient, an effective amount of one or more of the subject compounds and one or more nontoxic, pharmaceutically acceptable carriers or diluents can be used by persons of ordinary skill in the art. In addition, the pharmaceutical composition can comprise one or more of the bis-heterocycle compounds, e.g., a bis-indole, as a first active ingredient plus a second active ingredient comprising an anti-inflammatory compound known in the art. Such known anti-inflammatory drugs include, but are not limited to, the steroidal anti-inflammatory drugs and the non-steroidal anti-inflammatory drugs (NSAIDs).

In accordance with this invention, pharmaceutically effective amounts of a known anti-inflammatory agent and the bis-heterocycle compounds are administered sequentially or concurrently to the patienL The most effective mode of

administration and dosage regimen of bis-heterocycle compounds and anti-inflammatory agent will depend upon the type of disease to be treated, the severity and course of that disease, previous therapy, the patient's health status, and response to bis-indoles and the judgment of the treating physician. Bis-heterocycle compositions may be administered to the patient at one time or over a series of treatments.

Preferably, the bis-heterocycle, e.g., a bis-indole composition, and the second anti-inflammatory agent are administered sequentially to the patient, with the anti-inflammatory agent being administered before, after, or both before and after treatment with the bis-indole compound. Sequential administration involves treatment with the anti-inflammatory agent at least on the same day (within 24 hours) of treatment with bis-indole and may involve continued treatment with the anti-inflammatory agent on days that the bis-indole is not administered. Conventional modes of administration and standard dosage regimens of anti-inflammatory agents may be used (see Gilman, A.G. et *al.* [eds.] *The Pharmacological Basis of Therapeutics,* pp. 697-713, 1482, 1489-91 [1980]; *Physicians Desk Reference,* 1986 Edition). For example, indomethacin can be administered orally at a dosage of about 25-50 mg, three times a day. Higher doses can also be used. Alternatively, aspirin (about 1500-2000 mg/day), ibuprofen (about 1200-3200 mg/day), or conventional therapeutic doses of other anti-inflammatory agents can be used. Dosages of anti-inflammtory agents can be titrated to the individual patient.

According to one embodiment of this invention, the patient may receive concurrent treatments with the anti-inflammatory agent and compositions comprising bis-heterocycles, e.g., bis-indoles. For example, local, intralesional, or intravenous injection of bis-indoles is preferred (see Oilman *et al.*, *supra* at pp. 1290-91). The anti-inflammatory agent should preferably be administered by subcutaneous injection, subcutaneous slow-release implant, or orally.

Alternatively, the patient can receive a composition comprising a combination of one or more bis-indole compounds and an anti-inflammatory agent according to conventional modes of administration of agents which exhibit antibacterial, anticancer, antitumor, or anti-inflammatory activity. These include, for example, parenteral, subcutaneous, intravenous, or intralesional routes of administration.

The compositions used in these therapies can also be in a variety of forms. These include, for example, solid, semi-solid, and liquid dosage forms, such as tablets, pills, powders, liquid solutions or suspension, suppositories, injectable and infusible solutions. The preferred form depends on the intended mode of administration and therapeutic application. The compositions also preferably include conventional pharmaceutically acceptable carriers and adjuvants which are known to those of skill in the art. Preferably, the compositions of the invention are in the form of a unit dose and will usually be administered to the patient one or more times a day.

The compounds of the subject invention may also be administered utilizing liposome technology, slow release capsules, implantable pumps, and biodegradable containers. These delivery methods can, advantageously, provide a uniform dosage over an extended period of time.

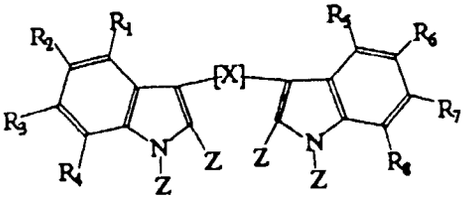
Examples of such carriers or diluents include ethanol, dimethyl sulfoxide, glycerol, silica, alumina, starch, and equivalent carriers and diluents. While effective amounts may vary, as conditions in which compositions are used vary, a minimal dosage required for anti-inflammatory activity is generally between 0.01 and 100 μg of the compound. To provide for the administration of such dosages for the desired therapeutic treatment, new pharmaceutical compositions of the invention will advantageously comprise between about 0.1% and 45%, and especially, 1 and 15% by weight of the total of one or more of the new compounds based on the weight of the total composition including carrier or diluent

Illustratively, dosage levels of the administered active ingredients can be: intravenous, 0.01 to about 20 mg/kg intraperitoneal, 0.01 to about 100 mg/kg; subcutaneous, 0.01 to about 100 mg/kg; intramuscular, 0.01 to about 100 mg/kg; orally 0.01 to about 200 mg/kg, and preferably about 1 to 100 mg/kg intranasal instillation, 0.01 to about 20 mg/kg; and aerosol, 0.01 to about 20 mg/kg of animal (body) weight.

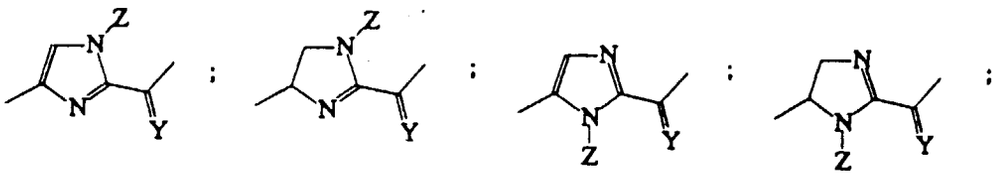
Once improvement of the patient's condition has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, may be reduced, as a function of the symptoms, to a level at which the improved condition is retained. When the symptoms have been alleviated to the desired level, treatment should cease. Patients may, however, require intermittent treatment on a long-term basis upon any recurrence of disease symptoms.

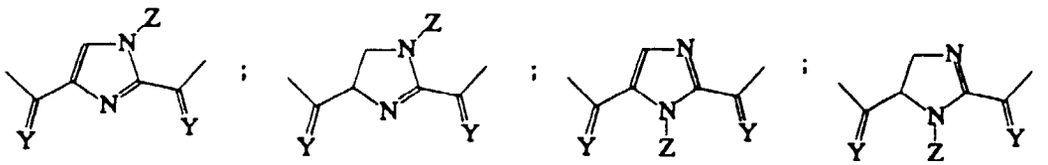
Claims

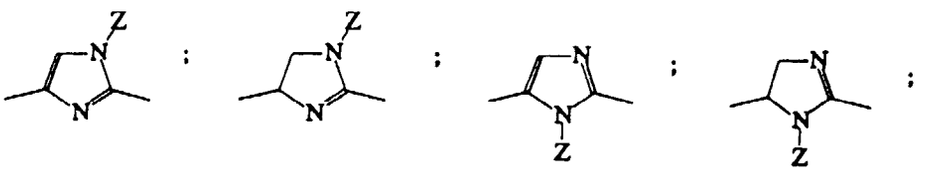
1. Use of a bis-heterocycle compound, for the manufacture of a medicament for inhibiting or controlling inflammation, wherein the bis-heterocycle compound has the formula

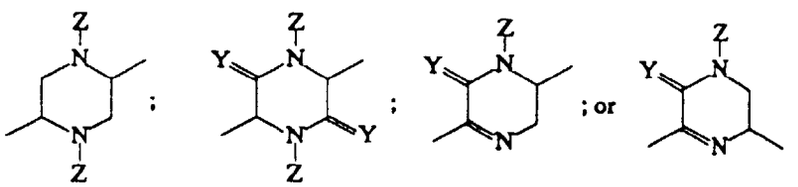


wherein X is



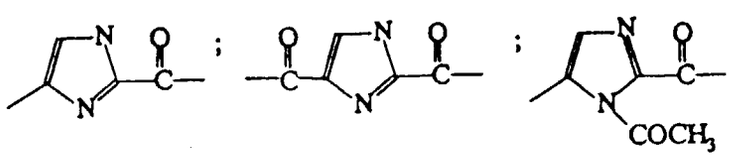


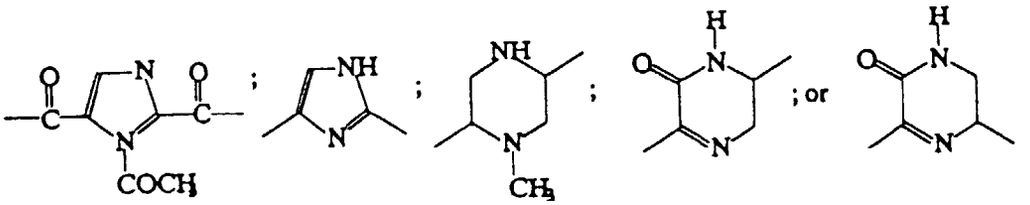




R_1-R_8 are the same or different and are selected from H, -OH, halogen, R, -OR_1, -OCOR and -O-R-phenyl;
Y is =O, or represents two groups which are the same or different, and are selected from H, -OH, -OR and -OCOR;
Z is independently selected from H, -R and -COR; and
R is C_{1-8} alkyl or C_{1-8} alkoxy.

2. Use according to claim 1, wherein X is





R_1, R_4, R_5, R_6 and Z are each H, at least R_2 and R_6, or R_3 and R_7, are each H, and R is C_{1-5} alkyl.

3. Use according to claim 1, wherein the bis-heterocycle compound is a hamacanthin.

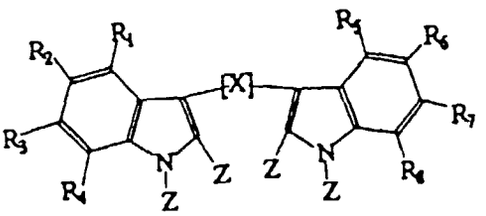
4. Use according to claim 1, wherein the bis-heterocycle is selected from topsentin, bromotopsentin, isotopsentin, hydroxytopsentin, deoxytopsentin, neotopsentin, neoisotopsentin, neohydroxytopsentin, homocarbonyltopsentin, topsentin monoacetate, topsentin diacetate, homocarbonyltopsentin monoacetate, nortopsentin A, nortopsentin B, nortopsentin C, dragmacidin, hamacanthin A and hamacanthin B.

5. A product containing a bis-heterocycle compound as defined in any preceding claim, and an anti-inflammatory agent as a combined preparation for simultaneous, separate or sequential use, for inhibiting or controlling inflammation.

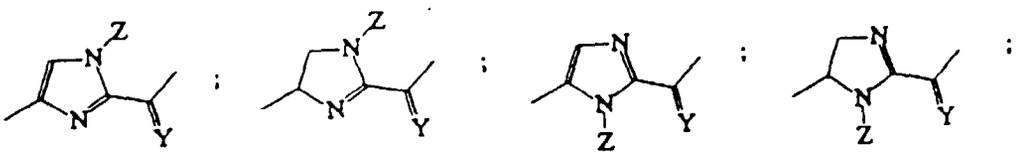
6. A compound selected from hamacanthin A, hamacanthin B, homocarbonyltopsentin, homocarbonyltopsentin monoacetate, and salts thereof.

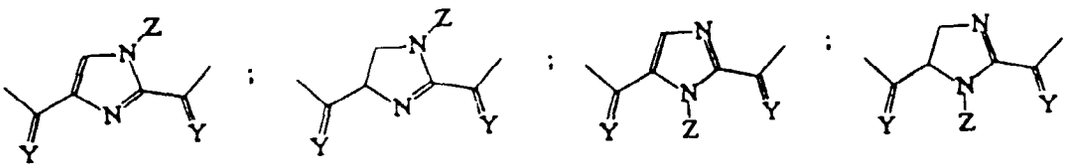
Patentansprüche

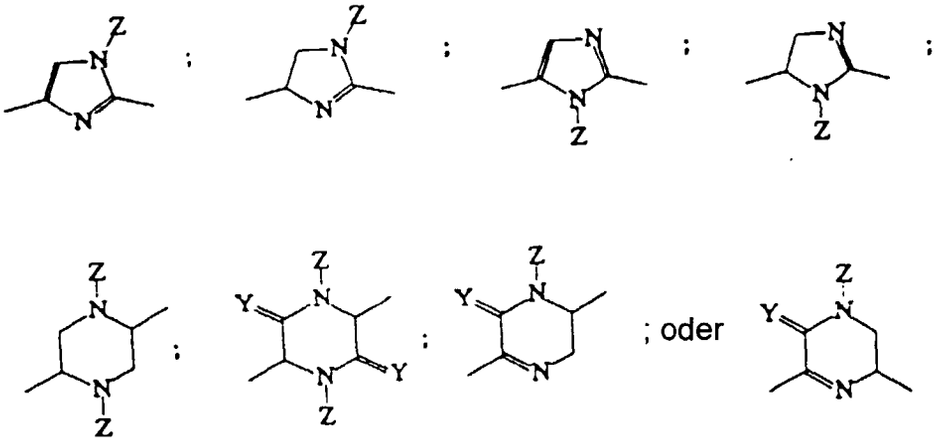
1. Verwendung einer bisheterocyclischen Verbindung zur Herstellung eines Medikaments zur Hemmung oder Kontrolle von Entzündungen, wobei die bisheterocyclische Verbindung die Formel



aufweist, worin X



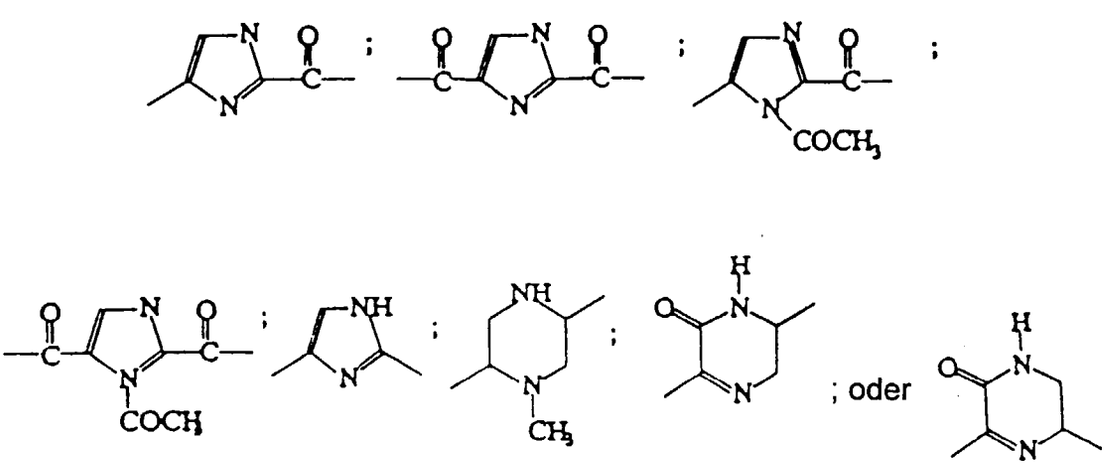




ist,

R_1-R_8 gleich oder verschieden sind und aus H, -OH, Halogen, R, -OR_1, -OCOR und -O-R-Phenyl ausgewählt sind;
Y = O ist oder zwei Gruppen repräsentiert, die gleich oder verschieden sind und aus H, -OH, -OR und -OCOR ausgewählt sind;
Z unabhängig aus H, -R und -COR ausgewählt ist; und
R C_{1-8}-Alkyl oder C_{1-8}-Alkoxy ist.

2. Verwendung nach Anspruch 1, worin X



ist, R_1, R_4, R_5, R_6 und Z jeweils H bedeuten, mindestens R_2 und R_6 oder R_3 und R_7 jeweils H sind, und R C_{1-5}-Alkyl darstellt.

3. Verwendung nach Anspruch 1, worin die bisheterocyclische Verbindung ein Hamacanthin ist.

4. Verwendung nach Anspruch 1, worin die bisheterocyclische Verbindung ausgewählt ist aus Topsentin, Bromotopsentin, Isotopsentin, Hydroxytopsentin, Desoxytopsentin, Neotopsentin, Neoisotopsentin, Neohydroxytopsentin, Homocarbonyltopsentin, Topsentinmonoacetat, Topsentindiacetat, Homocarbonyltopsentinmonoacetat, Nortopsentin A, Nortopsentin B, Nortopsentin C, Dragmacidin, Hamacanthin A und Hamacanthin B.

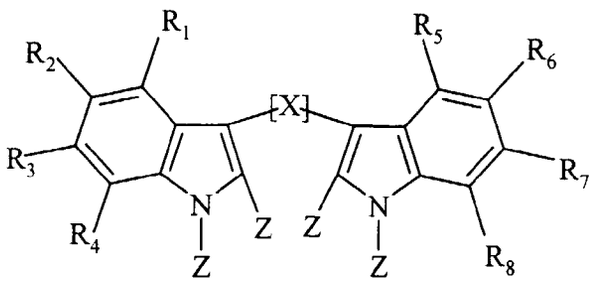
5. Produkt, enthaltend eine bisheterocyclische Verbindung wie in irgendeinem der vorhergehenden Ansprüche definiert und ein entzündungshemmendes Mittel, als Kombinationspräparat zur gleichzeitigen, separaten oder se-

quentiellen Anwendung zur Hemmung oder Kontrolle von Entzündungen.

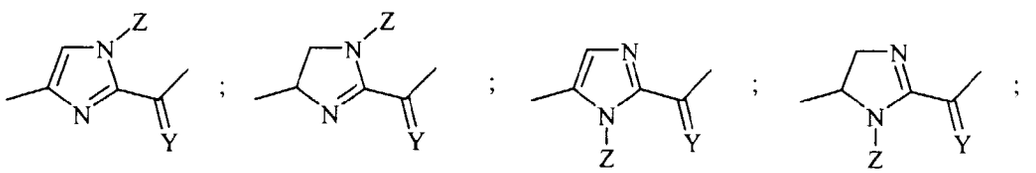
6. Verbindung, ausgewählt aus Hamacanthin A, Hamacanthin B, Homocarbonyltopsentin, Homocarbonyltopsentin-monoacetat und Salzen davon.

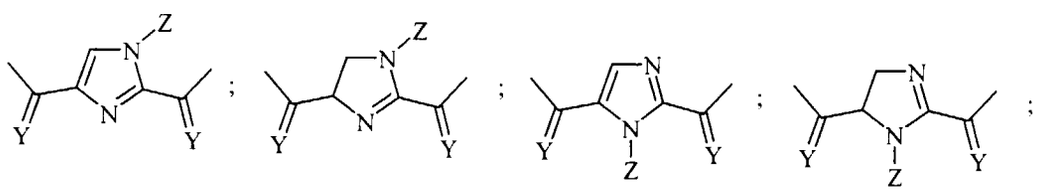
Revendications

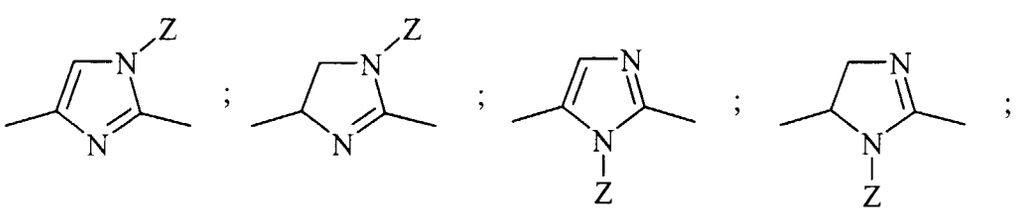
1. Utilisation d'un composé bis-hétérocyclique pour la préparation d'un médicament destiné à inhiber ou contrôler l'inflammation, où le composé bis-hétérocyclique a la formule

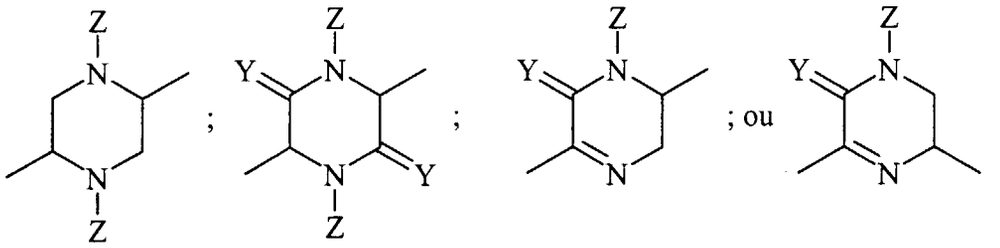


où X est



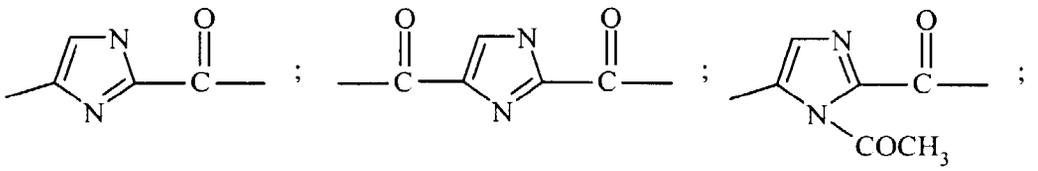


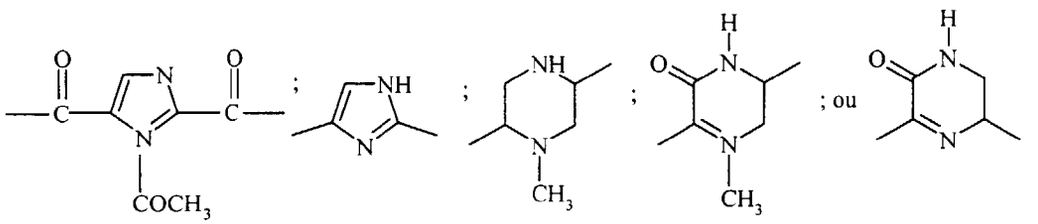




R_1-R_8 sont identiques ou différents et sont sélectionnés parmi H, -OH, un atome d'halogène, R, -OR_1, -OCOR et -O-R-phényle ;
Y = O ou représente deux groupes qui sont identiques ou différents et sont sélectionnés parmi H, -OH, -OR et -OCOR ;
Z est sélectionné indépendamment parmi H, -R et -COR ; et
R est un groupe alkyle en C_{1-8} ou alcoxy en C_{1-8}.

2. Utilisation selon la revendication 1, où X est





R_1, R_4, R_5, R_6 et Z sont chacun H, au moins R_2 et R_6 ou R_3 et R_7 sont chacun H et R est un alkyle en C_{1-5}.

3. Utilisation selon la revendication 1, où le composé bis-hétérocyclique est une hamacanthine.

4. Utilisation selon la revendication 1, où le bis-hétérocycle est sélectionné parmi la topsentine, la bromotopsentine, l'isotopsentine, l'hydroxytopsentine, la désoxytopsentine, la néotopsentine, la néoisotopsentine, la la néohydroxy-topsentine, l'homocarbonyltopsentine, le monoacétate de topsentine, le diacétate de topsentine, le monoacétate d'homocarbonyltopsentine, la nortopsentine A, la nortopsentine B, la nortopsentine C, la dragmacidine, l'hamacanthine A et l'hamacanthine B.

5. Produit contenant un composé bis-hétérocyclique selon l'une quelconque des revendications précédentes et un agent anti-inflammatoire sous forme de préparation associée pour utilisation simultanée, séparée ou séquentielle, pour inhiber ou contrôler l'inflammation.

6. Composé sélectionné parmi l'hamacanthine A, l'hamacanthine B, l'homocarbonyltopsentine, le monoacétate d'homocarbonyltopsentine et leurs sels.

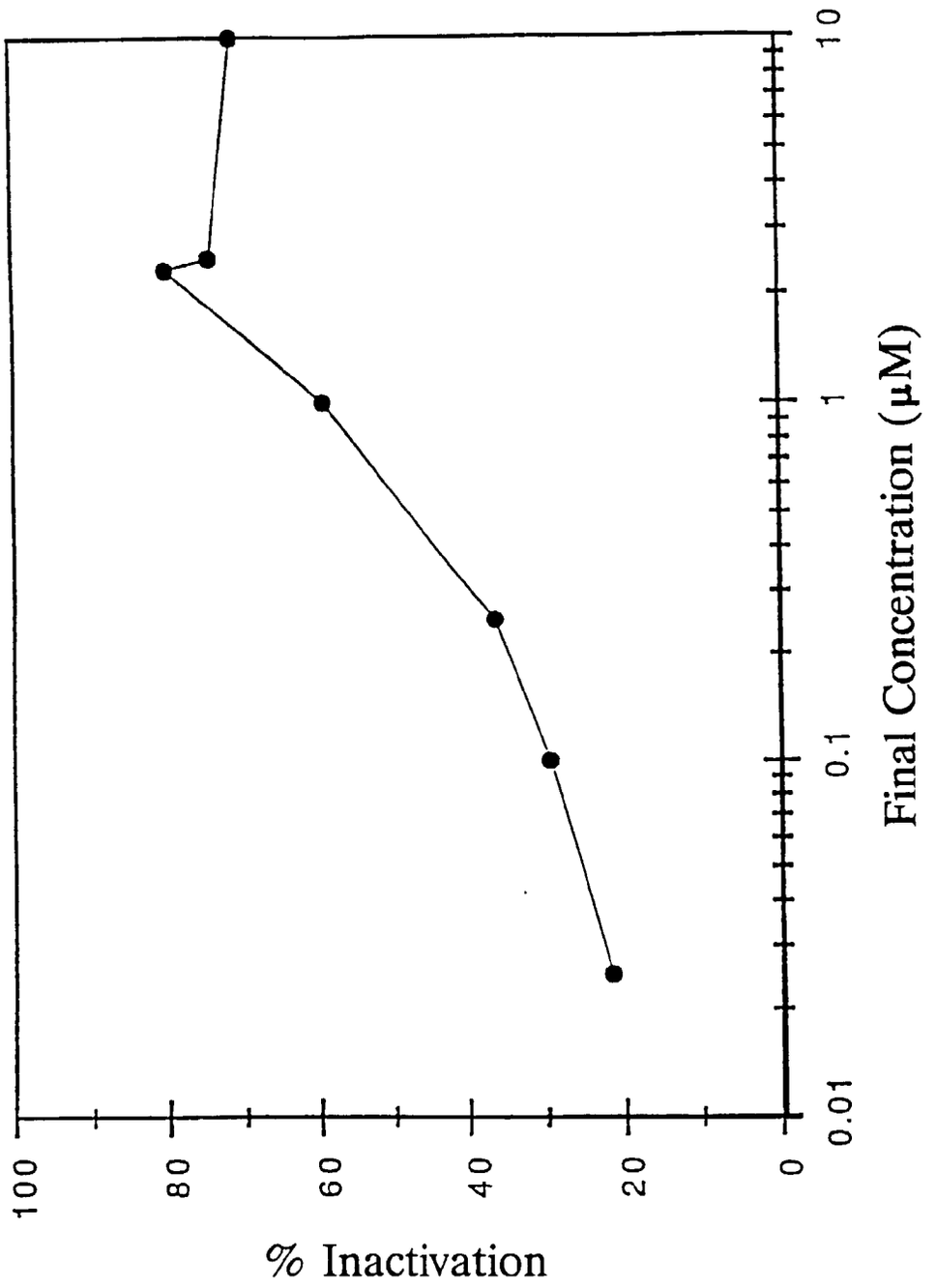

100
80
60
40
20
0
% Inactivation
0.01
0.1
1
10
Final Concentration (µM)

Figure 1

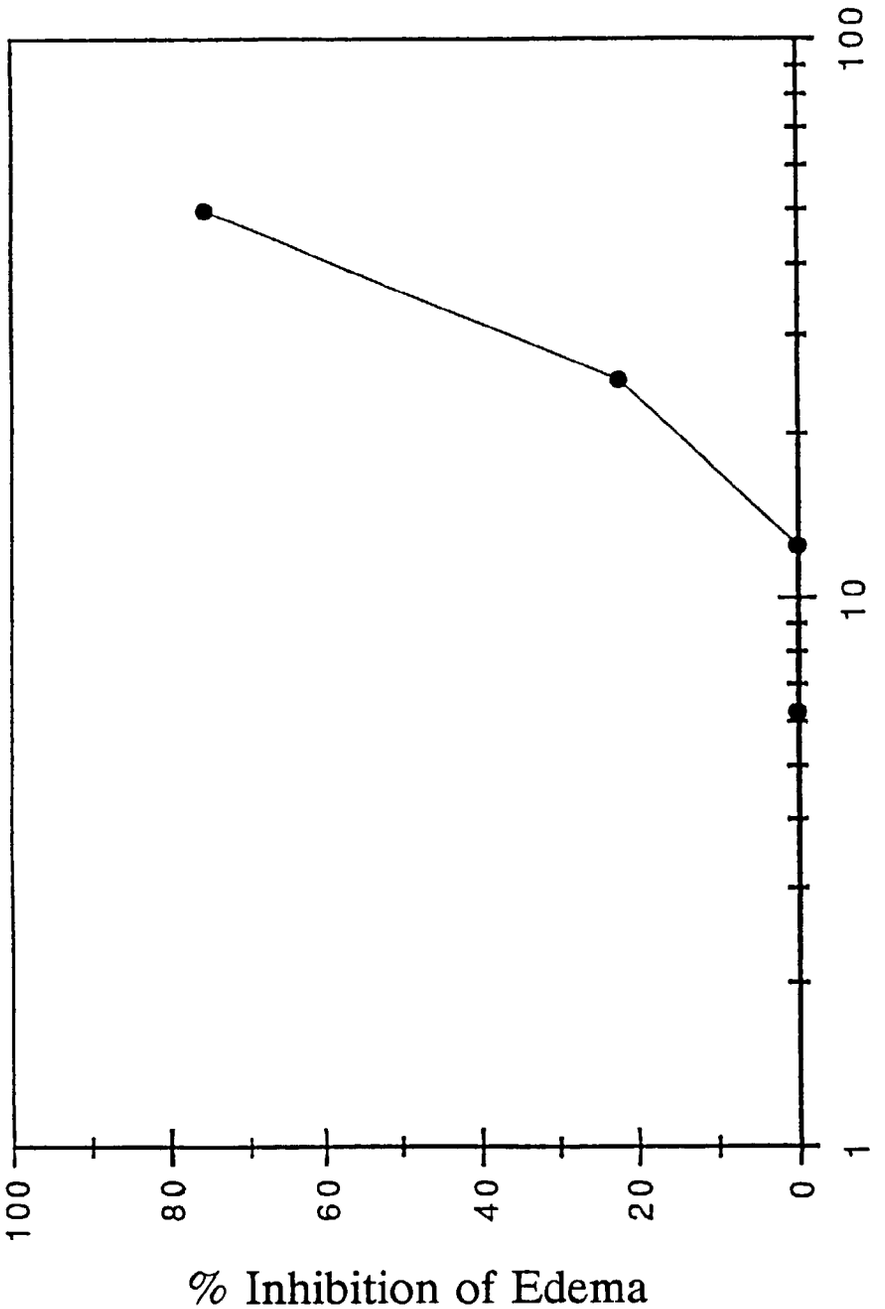


Figure 2

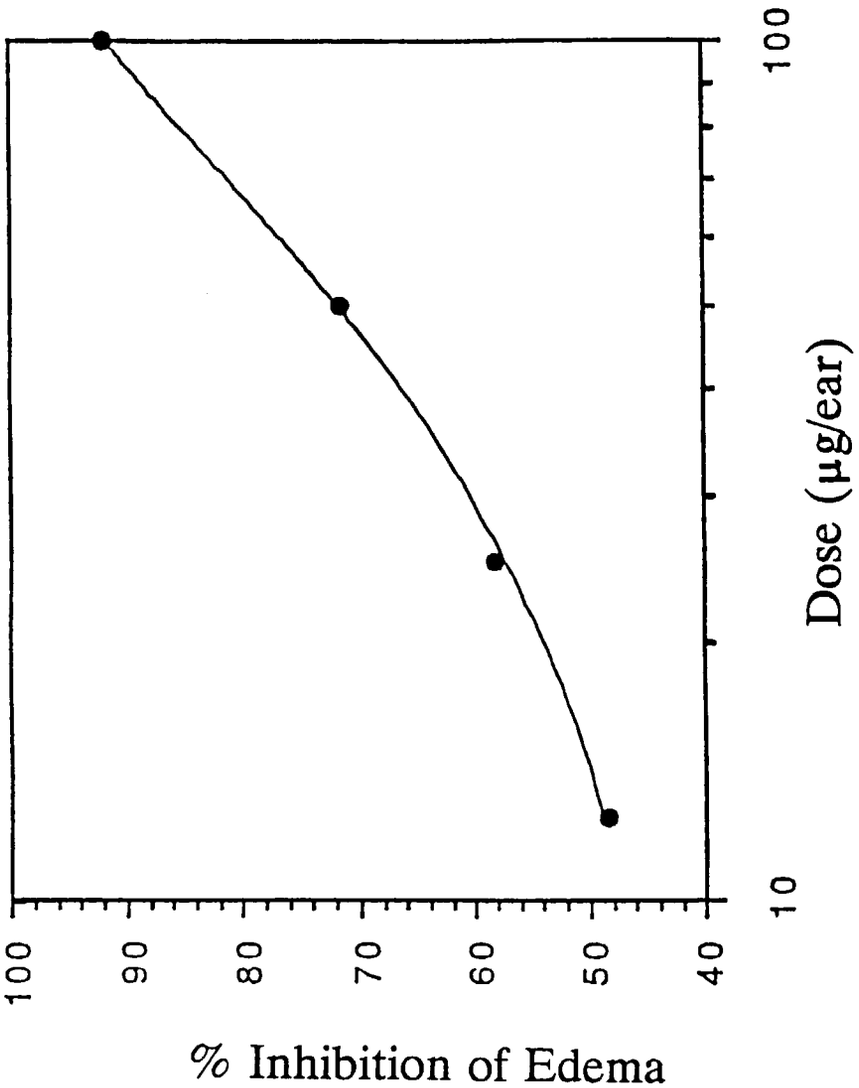


Figure 3